Plasmid Miniprep – Qiagen QIAprep Spin Miniprep Kit or our Homemade Miniprep Kit

Day 1:

1. Prepare 5 mL overnight cultures w/ antibiotic
   - Add 5 mL LB + 5 µL 1000x antibiotic to 15 mL culture tube
   - Pick a single colony from plate
   - Incubate in shaker overnight @ 37° C

Day 2:

1. Pellet 4.5 mL from 5 mL overnight cultures in a 1.5 mL Eppendorf tube
   - 3x – 1.5 mL increments
   - Centrifuge for 30 s. @ 13,200 RPM
2. Pour off supernatant and re-suspend cell pellet in 250 µL ice-cold Buffer P1
(**Technique:** Aspirate (draw & release) w/ pipet tip to help re-suspend cell pellet)
3. Add 250 µL of lysis Buffer P2, gently invert tube ~6 times to mix
(**Note:** Proceed to neutralization as quickly as possible)
4. Add 350 µL of neutralization Buffer N3, gently invert tube until thoroughly mixed
(**Note:** Vigorous mixing could potentially lead to genomic DNA contamination)
5. Centrifuge for 12 min. @ 13,200 RPM
(**Note:** This is a good time to pre-label collection tubes and 1.5 mL Eppendorf tubes)
6. Transfer the supernatant (~800 µL) to the corresponding mini-column w/ collection tube
7. Centrifuge for 1 min. @ 13,200 RPM, discard flow-through
8. Add 500 µL column wash Buffer PB
9. Centrifuge for 1 min. @ 13,200 RPM, discard flow-through
10. Add 750 µL of column wash Buffer PE
11. Centrifuge for 1 min. @ 13,200 RPM, discard flow-through
12. Centrifuge for 1 additional min. @ 13,200 RPM to remove residual column wash
13. Transfer the mini-column to corresponding sterile 1.5 mL Eppendorf tube
14. Add 40 µL of elution Buffer EB (or ddH_2O) directly to mini-column filter
(**Note:** Make sure surface area of filter is completely saturated)
15. Let stand for at least 1 min.
16. Centrifuge for 1 min. @ 13,200 RPM, remove mini-column
17. Measure DNA concentration (Nanodrop, Qubit, etc.)
18. Store @ -20° C